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EDITORIAL

EDUCATION FOR LEISURE

THE number of hours the American worker puts in on the job diminishes each passing year. A century ago, it was quite commonplace for many men to work seven 12 hour days a week with no vacation and with deductions from their wages should they be ill for a single day! It is difficult for one, today, to appreciate the slavery to one's job which existed then. In those days, one truly "lived to work" since little else found a place in the waking hours of the worker.

The many technological advances that have taken place in the factory, on the farm, and in the office have resulted in the need for fewer and fewer hours per week in order to earn a living and render that contribution to society required of all productive workers. The situation with the housewife is quite the same. With her myriad of automatic devices, much of the drudgery has been removed from house work and women have become emancipated from the kitchen slavery which once was their lot.

Today, we hear of automation and read of whole plants that are operated by a handful of men sitting in a control room where buttons and levers operate electronic devices and take over every detail of production and even quality control. There are some who fear automation is a threat to the worker but, like all technological advances, it may cause temporary dislocations but it cannot but help raise our standard of living. Hand in hand with this increased living standard must, of necessity, come a shorter work week, particularly in the field of manufacturing where so much can be done by so few in so short a time. A change in emphasis in the training of workers will, of course be necessary, for the time will come when we must have millions of electronics and mechanical experts since it is their skill which makes automation possible. In order to train these many thousands of experts, it would be well if their preliminary education were so patterned that they had a fundamental grasp of mathematics and physics when they left public school. The fact that these areas of

public education have been receiving less and less attention in our schools is a serious blunder, the full effects of which are just now beginning to be appreciated.

There is another aspect of public education, however, which is even more critical. Americans must be taught ways and means of occupying their leisure time in a manner which will benefit themselves and society and continue the progress of civilized man. There are some signs that the cultural trend of the average American is downward; at least, this is the viewpoint of some pessimists who view the American scene. If one judges the tastes and behavior of Americans in certain mass audiences as criteria, it would surely seem to be the case. Good music is almost impossible to hear today on the radio and really worthwhile moving pictures and television programs are a rarity as compared with the low grade entertainment seen on every hand. Mass entertainment in the United States is scaled at that level which appeals to the majority. This is probably the practical thing to do by those who control such things since almost all public entertainment is paid for by those who wish to sell their products. The tendency, however, is to degrade the taste of all down to this level, and this seems to be taking place. American culture today is heavily tinctured with juke boxes, pin-ball machines, tap-rooms, beer, soda-pop, candy bars, and other superficialities that are not to our credit. We seem much more concerned with flashiness and show than solid performance and even our skilled craftsmen take little pride in their work.

If this country is to occupy that position in world affairs to which we aspire, the rank and file of our citizens must present a picture to outside observers much different than we do at present. In many of the undeveloped countries which we now try to help, the people—poor as they are—have a culture and philosophy that transcends much of ours. To them, we are a crude, uncultured lot, wealthy it is true, but as barbaric as the Huns who sacked Rome.

Our world influence is the lesser of two reasons why some means needs to be found to raise our cultural level. If with increasing leisure we spend our time in pursuit of those avenues of pleasure now most popular in America, then our technological advances will be a curse rather than a blessing for our civilization will sicken and die on the vine. Such leisure can be a time to enjoy those mental and spiritual experiences which religion, art, music, and literature alone make possible. Man, if he is to achieve true greatness and be the true

son of God, must grow in intellectual stature until the joys of the spirit exceed those of the flesh.

Those who plan today the education of tomorrow's citizens must recognize the need for education for leisure. Courses alone are not enough for, to be effective, inspired teaching is indispensable. Truly, no greater challenge exists than for those who by dedicated teaching may well turn the tide of human destiny.

L. F. Tice

BRAND SUBSTITUTION *

By Newell Stewart **

THE story of highly trained professionals in every pharmacy adds much to the justification of public confidence but the substituting druggist is undermining our entire profession. It is unsound, economically, for those in the drug business to substitute. One pharmacist can hardly afford to compete with another pharmacist on the unknown terms of substitution.

The public interest is involved in brand substitution just as it is in drug substitution. The physician prescribes and the customer orders by brand names because he has confidence in the manufacturer. The production of drugs is a tricky business. Even the most reputable concerns can make mistakes in processing. The physician and the customer obviously want the fewest possible mistakes and if mistakes are made by the manufacturer, we want assurance that they will do their best to correct them. If the patient suffers a serious reaction from the medication, the physician should be in a position to notify the manufacturer's medical staff immediately. Giving an off-brand product short circuits this emergency procedure. If sales generated by the brand name advertising of the manufacturer are diverted to other concerns the incentive for further research is diminished and through this diminished research the public health is obviously affected.

The dispensing of any product differing in chemical nature, pharmaceutical formulation, color, odor, taste, appearance or *brand* from that specifically prescribed by the physician, without the express approval of the prescriber, is "substitution" and as such is unethical, unprofessional and illegal. The profession of Pharmacy generally has endorsed this thesis, and the Courts have always upheld it.

Pharmacy cannot equivocate when it interprets the word "substitution". When a *"prescription specialty"* is prescribed, it must be dispensed. The pharmacist's professional position depends upon this engagement. If the pharmacist calls the physician, he may then, and only then, dispense another brand. The responsibility for the quality, safety, efficacy and identity of the other brand is directly upon him.

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* Read before National Drug Trade Conferences, December 5, 1955.

** Executive Vice-President, National Pharmaceutical Council.

In a talk given by Dr. Albert H. Holland, Jr., Medical Director of the Food and Drug Administration, before the Fourth Annual Rutgers Pharmaceutical Conference, Dr. Holland stated: "There is another problem of growing magnitude with which your profession and the pharmaceutical industry must eventually come to grips. It is the pernicious practice of substitution. Several manufacturers estimate that they are losing as much as 20% of their sales volume on some items because of it. Everyone recognizes and appreciates that the average pharmacy must be selective in its stocking. Few physicians object to a legitimate request from the pharmacist to dispense another brand because he temporarily does not have that which was prescribed. That is not the problem. This problem is one of outright dishonesty. Buying a cheaper, often illegal, imitation of a good brand product, and unbeknownst to the doctor or patient, dispensing it in lieu of the brand product prescribed and, of course, at the higher price. There is only one discernible reason for this blight. One word—money. This is a problem for pharmacy. It is, in fact, a refutation of the years of painstaking work, and effort which so many have given to establish pharmacy on a sound professional basis. You may be assured that the manufacturers will continue to devise and study ways and means of effectively combating the practice of substitution. Where mislabeling, misbranding and danger to health is involved, appropriate governmental action can be anticipated."

No pharmacist denies that there are differences in quality between brands of a product. Where substitution flourishes, it has been found generally that reliability of the replacement brands generally yields to cost and the desire for increased profit, and quality suffers. Frequently, this in turn leads to the use of counterfeits of dissimilar composition.

No pharmacy act condones substitution, and in many there are sections which cover an offense of substitution. Some provide for penalty action, others for suspension or revocation of pharmacy permits, and still others for suspension or revocation of pharmacists' certificates. Boards of pharmacy are state enforcing agencies responsible to the public and there is always the danger of unfavorable publicity and undue emphasis whenever government is involved. Those boards achieving the greatest success in combating substitution have only been called upon to take action in the most flagrant cases.

Now the question arises, what should we do about it? We must reexamine our role as a pharmacist. Our job is to faithfully perform our duties as a public health servant. All else is secondary. If we put our main emphasis on satisfying the health needs of our people, and do it honestly and squarely, we will gain the respect we are entitled to. We must rededicate ourselves to this principle or we shall not achieve our rightful place in the health society.

I sincerely and most wholeheartedly believe that we pharmacists must begin to temper our practical nature with a little of the ideal. Deep within us we know what is right. Man-made laws are subject to change and revision but ethical laws and natural jurisprudence should remain unchanged throughout all time.

If the growing trend toward the practice of substitution is to be reversed, it will probably result only from the combined efforts of state boards of pharmacy, national, state, and local associations, colleges of pharmacy, honest manufacturers, and those individuals who recognize their professional obligations. Among these agencies and individuals, the state board of pharmacy is, by far, the most powerful and the most important. Only the state board has at its command a powerful weapon against unprofessional conduct, the threat of revocation of license. Again and again, it has been shown that those who are deaf to appeals to reason, honor or decency, who laugh at fines and similar penalties, are quick to respond to the threatened loss of their professional privileges. Revocation of license is a very severe penalty, one that should be invoked only for grave or repeated offenses, but its invocation, or even the threat of such action, can have quite wholesome results.

The position of the board of pharmacy with respect to the practice of substitution is not an easy one. It is charged with protection of public health, and substitution constitutes a potential, and often actual, threat to public health. It is, therefore, the duty of the board to proceed against those who substitute but, in attempting to do so, it may find itself at a disadvantage in several respects. The laws of some States, for instance, are so ambiguous and contradictory that the powers of the board are ill-defined and the members fear individual responsibility for their official acts. Another disadvantage lies in the costs of an enforcement program. Few boards have the staff or the funds to conduct such a program. They are hampered, too, by the difficulty of positively identifying some substitutes without knowledge of the "tracer" substance present in the genuine products, and manu-

facturers are understandably reluctant to divulge information concerning the secret substances.

These are real disadvantages but they can be overcome. State laws can be strengthened and clarified; board members can be protected from individual responsibility for official acts; funds can be secured for the investigations that will reveal violators; and the cooperation of honest manufacturers can be obtained.

Those of us who tread the commercial walks of life must consciously realize every day that, if we make a god of materialism and seek personal gain by deceit we are doomed to failure. The same is equally true of the pharmacies for which we work.

It is easy for all of us to agree that we have a legal approach to the substitution problem whereby our enforcement bodies may impose certain penalties upon those who are in violation of the law but it is more important for us to make the approach through our own honor, our integrity and the reputation we enjoy.

Toward this end it is no longer necessary for isolated groups to work alone. Just two years ago, in December, 1953, the National Pharmaceutical Council was created by a group of forward-looking manufacturers who were interested in the moral reappraisal of the profession of pharmacy. Our Council has given powerful support to the campaign to eliminate substitution and has developed a large amount of data on the subject of drug legislation in every state in the Union.

Substitution has been a problem with which we have all been concerned—it was a problem our fathers and, in fact, all of our pharmaceutical forebears have been concerned with but I believe we are beginning to see a definite lowering of the incidence in the recent past. Recent reports emanating from boards of pharmacy, manufacturers' surveys and individual findings have all pointed in this direction. Recognition of the problem has been highlighted by innumerable articles appearing in the pharmaceutical press both in this country and abroad. I was particularly interested in the response of a pharmacist of Tupper Lake, New York, who, in answer to a directive from the Department of Public Welfare of Franklin County, New York, demanding that cheaper, substituted medication should be dispensed for all welfare patients, made the following statement in the "Post-Standard" newspaper of Syracuse.

"I do not substitute any items in the prescription department without the express permission of the doctor and I do not intend to

change this practice, in violation of the law, to please any person or welfare official for any reason."

He further said that "the State Board of Pharmacy, a regulating board for all pharmacists and drug stores, defines "substitution" as the "dispensing of a different drug or brand of drug in place of the drug or brand of drug ordered or prescribed without the express permission of the prescribing physician."

It is well that pharmacists, throughout the country, have been alerted to the pernicious practice participated in by those who would sell their pharmaceutical birthright for a mess of cabbage. There have been those who have endeavored to create confusion in the minds of our practitioners by stating that one form of substitution is all right while another is all wrong. It reminds me of the story I heard down in the Carolinas recently about the confused state of the youngster who accidentally dropped his chewing gum in the hen house. Again I repeat that our job is to faithfully perform our duties as a public health servant. All else is secondary. We must do it honestly and fairly without equivocation in order to attain the goal we all aspire to reach.

BIOCHEMICAL ASPECTS OF BACTERIAL ENDOSPORE FORMATION AND GERMINATION [1]

By R. L. Stedman,* Sc. D.

PART I

Introduction

THE bacterial endospore has been recognized as a biological peculiarity since the initial discovery of this resistant form of life. Although a large number of studies on endospores have appeared in the literature during the past eighty years, most of our current knowledge of the biochemical aspects of sporulation and germination has been recently accumulated. In fact, almost all of the cited works on this subject have been published within the last seven years. It is the purpose of this review to examine and correlate the more significant findings on the physiology of spore formation and germination appearing within this latter period.

Apparently, the misconception that bacteria sporulate in an unfavorable environment for teleological reasons is still prevalent among microbiologists. The authors of two recent works on the modern aspects of microbiology (26, 49) have felt the necessity to reiterate the fallacy of such reasoning. As indicated by these authors and many others, sporulation is not an *a priori* survival mechanism. The transition from vegetative cell to spore probably requires more stringent environmental conditions than the comparable growth requirements for the species (26). In fact, many agents which have little or no effect on growth inhibit sporulation or germination (52). Perhaps the most recent explanation for the role of the endospore in the life of the organism is a reiteration of one of the oldest: Behring's hypothesis (1889) which states that the endospore simply represents another biological form in the life cycle of the sporulating species (52).

1. The opinions expressed herein are those of the author and are not necessarily similar to the opinions of the Department of the Navy.

* Industrial Test Laboratory, Philadelphia Naval Shipyard, Naval Base, Philadelphia 12, Pa.

Since complete reviews and studies summarizing the cytological changes involved during sporulation and germination are available (10, 25) no discussion of these findings shall be presented here. However, some mention should be made of the broad alterations in appearance and chemical content occurring during the transition from spore to vegetative cell, since investigations correlating chemical and cytological changes are cited in this report.

In general, the endospore appears as a highly refractile ovoid form which is resistant to the usual staining technics. The spore coat of the dormant spore is usually comprised of two layers: an outer, thin layer (probably of low refractive index) and an inner wall which is more dense and of a higher refractive index. The spore contains a large amount of "bound" water (probably similar to "water of hydration" and bound to macromolecular colloids), amino acids, peptides, hexosamine-peptide combinations, dipicolinic acid, and a high concentration of calcium (43). Little or no "free" water is present. The first phase ("Phase One") in the sequence of events called 'germination" consists of a loss of refractility of the spore with concurrent uptake of water causing a swelling (10, 43). A considerable portion of the internal spore solids are lost during this period (43) and the spore becomes penetrable to dyes. Following this, a break occurs in the spore coat ("Phase Two"). Ultimately, the new bacillus or "germ cell" emerges from the spore case ("Phase Three") and develops into a mature vegetative form ("Phase Four").* During the entire transition, progressive internal changes involving differentiation of nuclear material are observed; the significance of such changes has been reviewed elsewhere (9, 52).

Data of significance in the biochemical aspects of sporulation and germination have appeared for the following species**: *Bacillus subtilis* (3, 6, 14, 15, 22, 43, 50), *B. cereus* (1, 3, 9, 18, 11, 39), *B. anthracis* (1, 20, 21, 22, 45), *B. megaterium* (3, 9, 10, 22, 32, 33, 41, 43), *B. lacticola* (18), *B. mycoides* (12, 18), *B. globigii* (3, 34, 45) *B. polymyxa* (3, 30), *B. cereus var terminalis* (3, 19, 28, 29), *B. brevis* (6), *Clostridium botulinum* (23, 27, 52, 53), *C. perfringens* (53), *C. chauvei* (53), *C. acetobutylicum* (23) and an unidentified putrefactive anaerobe (53).

* With rare exceptions, endospore formation is confined to species of *Bacillus* and *Clostridium*.

** No attempt will be made in this review to discuss or note questionable taxonomic points which may arise in a perusal of the cited publications; all cited organisms are named in the manner employed by the original authors.

Methodology

Although a detailed consideration of methodology does not come within the scope of this report, it appears desirable to cite certain experimental details which are significant in the overall discussion or which may be of value to those initiating work on spore metabolism.

The growth conditions employed in preparing cells for sporulation and germination studies affect significantly the results obtained. For the production of large numbers of spores, aerated liquid medium is generally preferable to semisolid medium (*22*, 41). Oxygen tension and medium composition are of importance (3, 11, 41). Perhaps the most critical requirement for spore suspensions employed in biochemical studies is freedom from vegetative elements or debris ("clean" spores) and from traces of growth medium constituents. This is especially true in studies on the oxidative enzymes of endospores, in which small uptakes of oxygen may be significant.

The technic employed for the determination of the degree of germination occurring in spore suspensions is obviously a critical point in investigations of this type. One of the more accurate methods for determining percentage germination involves utilizing the loss in heat resistance as a measure of the number of spores germinated. Details of the method are given elsewhere (52), but, in general, the technic consists of heating a spore suspension previously exposed to the experimental variable affecting germination to a temperature which is lethal to germinating spores (but not to dormant spores) and plating the survivors. A control suspension is similarly treated, and a percentage germination calculated. This technic measures spores in all four phases of germination, but has the disadvantage of being quite detailed and time-consuming.

More recently, less cumbersome methods for quantative germination studies have been used. Uptake of stains such as methylene blue (3, 40), crystal violet-malachite green (51), malachite green—mercurochrome (53) and other dye combinations have been employed. Use of a staining procedure as a criterion of germination permits the detection of "Phase One", since loss of heat resistance and penetrability of dyes accompany the initial changes in spore refractility. Another type of technic for determining the extent of germination is based on changes in turbidimetric readings of cell suspensions at 610 mu (15, 40, 41) or with a 470-530 mu band filter (*32*). These methods provide a measure of the loss in refractility of the spore

during "Phase One" and have been correlated with acquisition of stain penetrability and loss of heat resistance. Other procedures which have served as criteria for germination are loss of labile catalase activity (3, 29) and initiation of respiration on glucose (32).

In most of the studies reported herein, "heat-shocked" or "heat-activated" spores have been employed. The use of such a procedure (exposure to 60°-85° C. for 5-60 minutes) eliminates the long lag usually encountered during germination of spores, even under the influence of an inducing chemical, and has been used as an experimental expedient. Further comments on this practice are given in detail below.

Induction of Germination

Rapid germination of bacterial endospores can be strikingly demonstrated by the addition of small amounts of various chemicals to an aqueous suspension of spores followed by incubation of the mixture at certain temperatures. In all cases incubation at ranges of room temperature suffices. For example, 10^8 "heat-shocked" spores per ml. (*B. cereus var terminalis*) will germinate in 5 minutes in an aqueous solution containing only 12 μM adenosine and 6 mM L-alanine per ml. at 30° C., pH 7.0 (47). "Heat-shocking" is necessary to obtain this rapid germination. Under these conditions, complete germination through all four of the above phases is not usually obtained, the transition proceeding merely to the loss of refractility stage with concurrent acceleration of oxygen uptake. For complete germination to the mature bacillus, minimal media containing glucose, inorganic salts and a nitrogen source such as amino acids (15) or natural materials (10) are required. Nevertheless, the role of small amounts of chemical substances in "triggering" the initiation of germination is well established.

A compilation of stimulatory agents appearing in the recent literature is given in Table 1. It should be noted that not all of these substances have been tested individually for germinating properties; in many of the studies, combinations were investigated and in these cases, an indication of a contribution to rapid germination by any component of the combination has been taken as a sign of positive inducing action in the preparation of this table, although the ability of the component to be active when present alone might have been insignificant. Also, no quantitative differentiation of germinating

effectiveness is included in the table, although marked differences in the germinating abilities of the various inducers were noted in the original studies. Nevertheless, the findings illustrate the diversity of chemical substances affecting germination of bacterial endospores and serve to provide a background for a discussion of the broad biochemical aspects of endospore germination.

TABLE 1

CHEMICAL COMPOUNDS AND SUBSTANCES STUDIED FOR THE CAPACITY TO INDUCE ENDOSPORE GERMINATION

Chemical compound or substance	*Effect on germination**	
	Slight or marked stimulation	*No effect or inhibition of stimulation*
Amino acids and related substances		
L-alanine	(3)[1,6] (10)[4] (15)[2] (19)[1] (22)[4,7,8] (23)[8,9] (30)[4,8] (40)[2] (47)[1]	(15)[2]
DL-alanine	(15)[2] (19)[1] (30)[1]	(32)[3]
D-alanine	(30)[1]	(21)[7] (22)[2]
β-alanine		(32)[3]
L-histidine	(15)[2]	
DL-valine	(14)[2] (15)[2]	(21)[7]
L-tyrosine	(14)[2] (15)[2] (21)[7] (22)[4,7]	
L-cystine	(15)[2]	
DL-cysteine		(21)[7]
L-tryptophane	(15)[2]	
Glycine	(15)[2]	(21)[7]
L-arginine	(15)[2] (23)[8,9]	
L-leucine	(15)[2]	

* Numbers in parentheses indicate reference number of report describing effect. Superscripts after parentheses refer to species studied, using following designations: 1—*Bacillus cereus* var *terminalis*, 2—*B. subtilis*, 3—*B. megaterium*, 4—*B. cereus*, 5—*B. polymyxa*, 6—*B. globigii*, 7—*B. anthracis*, 8—*Clostridium botulinum*, 9—*C. acetobutylicum*.

Chemical compound or substance	*Effect on germination**	
	Slight or marked stimulation	*No effect or inhibition of stimulation*
DL-methionine	(14)[2] (15)[2]	(21)[7]
DL-serine	(14)[2] (15)[2]	
DL-isoleucine	(14)[2] (15)[2]	
DL-phenylalanine	(14)[2] (15)[2] (23)[8,9]	
L-proline	(14)[2] (15)[2]	
L-hydroxyproline	(14)[2]	
DL-aspartic acid	(15)[2]	(32)[3]
L-aspartic acid		(32)[3]
Asparagine	(15)[2]	
L-glutamic acid	(15)[2]	
DL-ornithine	(15)[2]	
Casamino acids	(14)[2]	
Carbohydrates and compounds or substances related to carbohydrate metabolism		
Glucose	(3)[1] (10)[3,4] (15)[2] (19)[1] (40)[2] (41)[3]	
Caramelized glucose**	(16)[2]	
Maltose	(41)[3]	
Caramelized maltose	(16)[2]	
Galactose (intact and caramelized)	(16)[2]	
Lactose (intact and caramelized)	(16)[2]	
Sucrose (intact and caramelized)	(16)[2]	

** Glucose stimulation in (15)[2] was subsequently shown to be due to caramelization of glucose (16).

Chemical compound or substance	*Effect on germination**	
	Slight or marked stimulation	*No effect or inhibition of stimulation*
Fructose		(16)[2]
Caramelized fructose	(16)[2]	
Mannose (intact and caramelized)	(16)[2]	
Lactate	(32)[1]	(16)[2] (41)[3] (19)[1]
Lactate & NH_3		(21)[7]
Pyruvate	(16)[2] (30)[1]	(19)[1]
Pyruvate & NH_3		(21)[7]
Acetate	(32)[3]	(16)[2]
Formate	(32)[3]	
Propionate	(32)[3]	
Citrate		(16)[2]
α-Ketoglutarate		(16)[2]
Succinate	(16)[2]	
Fumarate	(16)[2]	
Malate	(16)[2]	
Oxalacetate	(53)[8]	
Glycerol		(19)[1]
Glycerol phosphate		(41)[3]
CO_2	(53)[8]	
Nucleic acids and compounds or substances related to same		
Ribonucleic acid	(20)[7]	
Desoxyribonucleic acid		(20)[7]
Guanylic acid		(20)[7]
Adenine-cytosine-dinucleotide	(20)[7]	
Guanosine-5-phosphate		(30)[1]

Chemical compound or substance	*Effect on germination**	
	Slight or marked stimulation	*No effect or inhibition of stimulation*
Xanthosine-5-phosphate		(30)[1]
Adenosine triphosphate	(20)[7]	
Adenylic acid (yeast)	(20)[7]	
Adenosine-5-phosphate		(30)[1]
Inosine	(30)[1]	
Xanthosine	(30)[1]	
Cytidine		(30)[1]
Guanosine	(20)[7] (47)[1] (30)[1]	
Uridine		(30)[1]
Adenosine	(3)[1] (10)[3,4] (19)[1] (21)[7] (22)[7] (30)[5] (31)[3]	(15)[2] (30)[6]
Guanine		(20)[7]
Adenine		(20)[7]
Ribose		(41)[3]
Ribose & adenylic acid	(20)[7]	
Ribose & adenine		(47)[1] (30)[1]
Yeast extract	(20)[7]	
Inorganic ions		
Manganese	(32)[3] (31)[3]	
Magnesium		(32)[3]
Iron (bi-and trivalent)		(32)[3]
Copper		(32)[3]
Cobalt	(32)[3]	
Zinc	(32)[3]	
Chloride	(32)[3]	

Chemical compound or substance	*Effect on germination**	
	Slight or marked stimulation	*No effect or inhibition of germination*
Fluoride		(32)[3]
Nitrate	(32)[3]	
Orthophosphate and pyrophosphate	(3)[1,2,3,4,5,6]	(30)[3]
"Proper ionic balance"	(32)[3]	

The effectiveness of chemical agents in producing rapid germination of spores is apparently a function of species-specificity, at least among *Bacillus* species, although differences in the qualitative and quantitative requirements among various organisms are not striking. For example, one hundred percent germination of *B. cereus* var *terminalis* spores can be attained in 60 minutes by a combination of 6 μM L-alanine, 12 muM adenosine and 67 mM pyrophosphate (3). On the other hand, spores of *B. globigii* are germinated under these conditions by a mixture of 12 μM L-alanine, 10 μM glucose and 30 μM orthophosphate. Complete germination of certain other *Bacillus* species cannot be demonstrated with any combination of the above agents, the maximum percentage of germination of *B. subtilis* and *B. polymyxa* spores being 35% in each case. Such species differences may be a reflection of minor variations in metabolic pathways, coenzyme or enzyme concentrations, or other kinetic factors in the various organisms.

The effect of L-alanine on the germinative process has been studied more intensively than any of the other agents. In all but a single instance (described in detail below), a striking stimulation of germination occurs with this amino acid. However, the stimulation is very sensitive to the D-form of the acid in some instances. Hills (21) was the first to observe an inhibition of L-alanine induction by D-alanine in *B. anthracis* and *B. subtilis:* ratios of approximately one part D-form to thirty parts L-form produced an inhibitory mixture in which anthrax spores did not germinate. Subsequent work on this antagonism between enantiomorphs in other species has indicated a species-difference in regard to the degree of inhibition obtained (19). In the case of *B. cereus* var *terminalis,* equimolar quan-

tities of the D- and L-forms, when added as the individual enantiomorphs, give a lower rate of germination than that obtained with L-alanine alone: the D- and L-mixture permits 90% germination of the spores when the L-form gives complete germination. Evidence of enantiomorphic antagonism in other amino acids is limited to the observation that suppression of the stimulatory effect of L-tyrosine on *B. anthracis* is not observed with DL-tyrosine (21). The degree to which D- and L-antagonism has contributed to the reported failure of some DL-forms to produce high degrees of germination (15) is not known, although many racemic amino acids are, nevertheless, highly active. Recently, a report has appeared in which germination of *B. cereus* var *terminalis* spores under certain conditions pertinent to enantiomorphic suppression is described (30). On pre-heating the spores at 60° C. for 15 minutes in the presence of D-alanine, a degree of germination is observed equivalent to that obtained with L-alanine (without pre-heating) after subsequent incubation at room temperature with adenosine. However, no explanation of this finding was presented.

Data on the germinating capacities of other amino acids show that distinct qualitative and quantitative differences are observed when various species are studied under diverse conditions. L-Tyrosine contributes to the germination of anthrax spores by L-alanine and adenosine, and compounds structurally related to tyrosine (tyramine and diiodotyrosine) also show this effect (22). Of fourteen amino acids tested, germination of *B. subtilis* spores was obtained with DL-alanine, L-tyrosine, DL-cystine and DL-tryptophane in the presence of adenosine but not in its absence (44). In another work, glycine, DL-methionine, DL-cysteine and DL-valine have been shown to inhibit the germination of *B. anthracis* spores but thirteen other amino acids had no effect on germination. In combination with glucose, each of eight amino acids germinated 20-100% of *B. subtilis* spores in the investigation of Hachisuka, et al. (14). Later work (15) by this group elucidated the key role of glucose in the stimulation by a number of amino acids. In the absence of glucose, the maximum percentage germination of *B. subtilis* spores attained with amino acids was 39% but twelve of the twenty amino acids gave no germination. However, in the presence of glucose, all amino acids except one gave measurable germination, although the percentages varied from 3% (L-cystine) to 96% (DL-isoleucine). Of more importance, the responses of the compounds permitted the classification of amino acids into four cate-

gories based on differences in the effects on germination and growth. It was noted that some amino acids, such as DL-serine and DL-methionine, cause the appearance of translucent areas in spores but fail to promote development through the next three phases of germination. Others, such as DL-alanine and DL-isoleucine, initiate the complete germination process and permit growth of the new bacillus. Surprisingly, L-alanine was found to be relatively ineffective in initiating germination (in the presence of glucose and adenosine) although the DL-form promoted both germination and growth as noted above.

In general, the findings on amino acid stimulation of spore germination show that these compounds undoubtedly play a key role in the transition from spore to bacillus. Although conflicting reports on the relative effectiveness of certain amino acids have appeared, the wide variations in experimental conditions may be responsible, at least in part, for the observed discrepancies. Differences in species requirements may also be contributing to the findings.

On the basis of available data, the role of alanine appears to be critical in the initiation of germination not only because it has been found stimulatory in so many instances, but also since an enzyme capable of acting on the L- or D-form of the acid has been found in dormant spores (47). Alanine racemase is present in higher concentrations in spores than in vegetative cells, which is quite unusual since dormant spores are, in the main, relatively inactive. Although alanine racemase occurs in the spores of many species of *Bacillus*, attempts to relate enzymatic activity directly with germination have been unsuccessful (3).

The role of alanine in inducing rapid germination is not known. However, it appears that alanine acts in an autocatalytic manner since the quantitative uptake of C^{14} tagged DL-alanine by spores during germination is almost undetectable by current analytical technics (19). Except for racemization, enzymatic reactions for the transformation of alanine (oxidative deamination and transamination) have not been detected in dormant spores.

As shown in Table 1, a number of other chemical substances have been studied for an effect on endospore germination. Briefly, these can be divided into nucleic acids and degradation products thereof, carbohydrates and intermediates or products of carbohydrate degradation, and inorganic ions.

Interest in compounds related to nucleic acid metabolism has arisen as a result of the initial observation by Hill (20) that the

germinative properties of yeast extract can be attributed to its adenosine content. Adenosine has been studied more intensively than any of the other nucleosides and nucleotides, as seen in Table 1, and has been found stimulatory for a number of species of *Bacillus*. *B. globigii* does not require adenosine for optimal germination in the presence of L-alanine, as noted above, but a number of other species are affected by this nucleoside. A conflict exists on the effectiveness of adenosine in stimulating germination of *B. subtilis* (6, 15) but strain-specificity may explain the differences in findings (15).

Two recent reports have elucidated further significant properties of systems in which endospores are germinated in the presence of adenosine (30, 31). By pre-heating the spores for various periods at 60° C., germination by adenosine alone equivalent to that obtained with L-alanine and adenosine combinations can be demonstrated, although extensive pre-heating and subsequent incubation at room temperature are required (30). Also, significant adenosine cleavage by dormant spores occurs under conditions promoting germination (31); however, a direct correlation between nucleosidase activity and germination cannot be demonstrated.

A considerable number of other nucleic acid degradation products have been examined for germinative properties. Among the ribosides, inosine, guanosine and xanthosine have been shown to be active inducers of germination; the pyrimidine ribosides which have been studied, are, on the other hand, not effective in this capacity. Although addition of phosphate to the 5-position in the guanosine and adenosine structures results in the loss of germinative properties, yeast adenylic acid containing phosphate in the 2- or 3-position, is an active inducer. The hydrolytic degradation products of adenosine, either individually or in combination, are not stimulatory. This is, perhaps, to be expected since the action of the spore enzymes which cleave nucleosides and nucleotides, including adenosine and many of the above derivatives, cannot be correlated directly with the germinative process, as noted above. Ribonucleic acid (yeast) is an active inducer but desoxyribonucleic acid shows no effect. This confirms the importance of ribonucleic acid in the germinative process which has been demonstrated in biochemical studies discussed in Part II of this report.

Among the compounds related to carbohydrate metabolism, glucose has been investigated most intensively as a stimulant of germination. Again, differences in the responses of *Bacillus* species are evident. Significant germination in the presence of glucose alone

has been shown with *B. megaterium* (41) and *B. globigii* (3), but no stimulation under these conditions can be observed with *B. subtilis* (15). In most instances, glucose has been employed in combination with other agents, such as L-alanine and adenosine, in inducing germination. A recent report (16) shows that an experimental variable previously unrecognized might have influenced the findings of a large number of studies conducted prior to 1955. In this report, it was demonstrated that autoclaving of glucose and an amino acid produces a stimulatory mixture which is not evident when each component is autoclaved separately and then mixed aseptically, confirming in part the observation of Powell (22) that glucose stimulation is not due to products formed during autoclaving of the sugar alone. This stimulation can be duplicated when various caramels prepared from heating crystalline glucose are added to an amino acid without autoclaving any of the ingredients. Also, caramels of maltose, lactose, fructose and sucrose are effective although the intact form of some of these carbohydrates are not strongly stimulating in some cases. The mechanism of this stimulation by caramels is not known, but it has been suggested (16) that they do not serve as an energy source. (Further comments on this point are given in the *Discussion* of this review).

In the main, studies on the effect of intermediates and end products of glucose degradation have been lacking. Available data indicate that the degree of induction encountered with such compounds as L-alanine, adenosine and caramelized glucose cannot be obtained with certain tricarboxylic acid cycle intermediates and glycerol phosphate. The relative germinating capacities of these Krebs cycle intermediates (in the presence of asparagine) is of interest: citrate and α-ketoglutarate give no stimulation, but succinate, fumarate and malate show 6, 7 and 10 percent germination, respectively (15). This pattern of stimulation bears a resemblance to the order of permeability of these subtrates to many bacterial species. Citrate and α-ketoglutarate are frequently not oxidized by intact cells due to impermeability; succinate, fumarate and malate are usually oxidized under these conditions when the cycle is operative. Perhaps the failure of these subtrates to penetrate the spore coat may have contributed to the above findings on spore germination.

Although pyruvate has shown very slight (4%) and lactate no induction of germination using *B. subtilis* spores (15), a marked stimulation is observed with both compounds if the spores are pre-

heated with these subtrates (30). This is similar to the effect obtained with D-alanine noted above. The mechanism of this induction is not known, but participation of transamination is doubtful unless a "heat-activated" enzyme is involved (*vide infra*). Certain inorganic ions and mixtures of salts have also been shown to influence the germination of a number of *Bacillus* species. The most significant of these is the manganous ion. Possibly manganese functions in an activating capacity in certain enzymatic reactions since Levinson and Sevag (33) have shown that Mn accelerates the proteolytic activity of *B. megaterium* spore homogenates. Manganese also has a stimulatory effect on the rate of sporulation of other *Bacillis species* (6, 51).

In general, the data on the stimulation of spore germination by chemical agents other than amino acids, show wide variations in responses. Certain compounds and mixtures, particularly specific ribosides and caramelized or intact mono- and disaccharides, exhibit a marked stimulation. The findings do not permit a concise indication of the specific biochemical reactions or types of metabolic sequences involved, since a large number of hypothetical interactions involving nucleic acid, protein and carbohydrate metabolism can be envisioned by imaginative speculation based on the above data. However, findings of certain studies described in Part II of this report are of interest in this regard, and the general biochemical reactions shown to be involved in sporogenesis and germination will be discussed in some detail therein.

Editor's Note—Part II of this paper will be published in a subsequent number of this journal.

THE APPLICATION OF THE PALISADE NUMBER METHOD TO THE QUANTITATIVE DETERMINATION OF SPEARMINT AND PEPPERMINT WHEN IN POWDERED ADMIXTURE

By Harold I. Silverman * and Marin S. Dunn * *

THE Labiatae drugs, spearmint and peppermint, resemble each other quite closely botanically, and because they are frequently grown in neighboring areas, the problems of adulteration (1), and contamination (2, 3) may arise. Also they may be used as condiments and it may be necessary for a microscopist to determine the purity of the powdered form of each of these drugs (4).

Taking the above facts into consideration, the following method has been developed to determine the approximate percentages of the two leafy drugs when powdered and in admixture. This method has been employed in our laboratories seven times on admixtures of authenticated commercial samples of these drugs and it has proven itself to be of value. These mixtures were made both by the authors and other members of the College Staff by combining these drugs after having first weighed each one separately on a torsion prescription balance.

Basically, palisade numbers (5) are obtained in properly cleared and stained material for each epidermal-palisade fragment observed in a systematic microscopical survey of a quantity of powder. From these numbers, it is possible to total the identifiable units of spearmint and peppermint in a given quantity of admixture (see later), and thus determine the approximate proportion.

Method

Each portion of epidermal-palisade tissue is counted as one unit as long as a sufficient number of palisade cells remain attached to the epidermal cells to enable the microscopist to identify the botanical

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source of this tissue. Counts of palisade cells are made using a standardized ocular net of Howard ruling in the 10× ocular of a compound microscope along with a 43× objective. Both spearmint and peppermint are recognized by measurement of the size of the palisade cells (5).

The method was developed on powdered material sufficiently fine to pass entirely through a number 80 sieve.

A representative portion (ranging from one to two grams) is removed from the admixture by quartering, weighed on a torsion prescription balance and ground to the fineness of a number 80 powder. The grinding apparatus used in this study was a Centrifugal Wet Mill manufactured by E. H. Sargeant and Company. This mill we find has several advantages, the most important of which is that there is no appreciable sample loss during the grinding period. Also, quantities as small as one gram are satisfactorily comminuted. While a mortar can also be used, there is the problem of sample loss due to air currents, and greater tissue destruction. The material to be ground is placed into a beaker, suspended in carbon tetrachloride and the grinding elements of the mill immersed. At the completion of the grinding period, the solvent is removed from the powdered product by vacuum filtration using a Büchner funnel, and the powder is air dried. The resultant powder is then passed entirely through a number 80 sieve, regrinding if there are tailings, until 100% passage occurs.

The powder is then treated with solvent ether (at the boiling point) in order to remove any remaining fat, oil or waxes that were not removed by the carbon tetrachloride. Also, most of the remaining chlorophyll is removed with the ether so that the resultant powder contains relatively little pigment. The sample is placed in a 50 ml. centrifuge tube and ether is added to fill approximately two-thirds of the tube. This mixture is then heated gently on a water bath until the ether boils, with care being taken to prevent spashing. After approximately five minutes of heating, the material is centrifuged at 1500 r.p.m. for five minutes. At the end of this period the ether is poured off through a Büchner funnel, which serves to trap any material that may accidentally be poured out of the tube. This process of washing with ether is repeated until the color of the ether solution changes from a green to a light yellow.

After all of the ether is removed, the powder is then stained, to facilitate counting. The powder is allowed to remain in the centrifuge

tube and to it is added 7 ml. of a 0.5% solution of Bismarck Brown, the mixture well stirred and allowed to stand for five minutes. To it is now added one drop of a surface-active agent (code No. G-2132, Atlas Corporation), which increases the penetration of dye and produces uniformity of staining. The mixture is again stirred and allowed to stand for a second five minutes. At the completion of the second period of standing, 3 ml. of a 10% solution of potassium hydroxide is added, and this serves to increase the brilliancy of the stained material. The mixture is again well stirred and allowed to stand for a third five minutes. At the end of this third standing period, distilled water is added to fill two-thirds of the centrifuge tube and the contents centrifuged for five minutes at 1500 r.p.m. The supernatent liquid is poured off through a Büchner funnel, and the water-washing process, as described, is repeated until the color of the wash liquid has changed from dark brown to tan. This water-washing process is carried out in order to remove the excess stain and to arrest the staining process at the proper level. Any material that may have been accidentally poured over onto the funnel is washed back into the centrifuge tube with a stream of water from a wash bottle.

The material in the centrifuge tube is now poured into a suitable storage bottle with a screw top, any material remaining in the centrifuge tube is washed out into the storage bottle with a stream of water from a wash bottle.

The material is now diluted with distilled water in the proportion of approximately 120 ml. per gram of powder, basing the weight of powder on the sample as originally taken for analysis. This dilution factor serves to produce microscopial fields of view which contain a convenient number of histological elements for counting, so that counting is easily and quickly accomplished.

The suspension is thoroughly shaken and portions quickly removed for the microscopical analysis. A long-stemmed uncalibrated dropper is partly filled with the suspension from the bottle and several drops immediately rejected back into the bottle using a rubber bulb to extrude the liquid. One drop is then placed on one chamber of a Levy Leucocyte Counter (Fuchs-Rosenthal double ruling), with the dropper being held in a vertical position. The remainder of the suspension in the dropper is redeposited into the bottle. The suspension is again thoroughly shaken, the dropper refilled, and the process repeated to fill the second chamber of the slide. The cover glass is carefully placed into position in order to avoid air bubbles and uneven

distribution of material over the counting area. If either or both occur, the procedure is repeated until these difficulties are remedied.

Using the 43× objective, the epidermal-palisade particles are identified and counted with the aid of the ocular net (5). The elements are counted in the entire ruled counting areas (using the guide lines to insure complete coverage) of both sides of the leucocytometer using hand counters as an aid in recording. A total of eight separate chambers are counted for each mixture (four slides, each with two counting chambers).

The counts for each of the two drugs are totaled. These totals are then combined to result in a final total which represents all of the epidermal-palisade units counted for a particular admixture (Table 1).

TABLE 1

COUNTS ON ADMIXTURES

1	2	3	4	5	6	7	8	9	10	11	12	13	14
		Leucocytometer counts											
No.	*Mixture*	1		2		3		4		*Total*	*Calculated %*	*Actual %*	*% Variation*
		A	B	A	B	A	B	A	B				
1	Spearmint	19	27	19	16	28	22	16	16	163	50.5	50.0	+ 1.0
	Peppermint	22	25	16	17	21	20	25	14	160	49.5	50.0	— 1.0
2	Spearmint	12	17	5	14	7	12	8	9	84	34.0	39.8	— 14.6
	Peppermint	21	30	12	22	28	22	14	14	163	66.0	60.2	+ 9.6
3	Spearmint	0	6	6	3	4	5	2	2	28	13.4	10.0	+ 34.0
	Peppermint	20	15	31	32	21	27	19	26	181	86.6	90.0	— 3.8
4	Spearmint	4	2	5	0	1	2	3	2	19	11.4	10.0	+ 14.0
	Peppermint	21	23	22	19	13	16	13	20	147	88.6	90.0	— 1.6
5	Spearmint	20	30	15	22	25	27	18	20	177	66.8	70.0	— 4.6
	Peppermint	10	8	10	15	9	13	13	10	88	33.2	30.0	+ 10.7
6	Spearmint	23	20	23	14	27	20	27	18	172	50.6	50.0	+ 1.2
	Peppermint	18	13	19	21	25	28	25	19	168	49.4	50.0	— 1.2
7	Spearmint	30	34	16	28	15	17	25	27	192	46.8	50.0	— 6.4
	Peppermint	30	39	23	31	19	19	27	30	218	53.2	50.0	+ 6.4

To determine the approximate percentage of each ingredient in the mixture, each drug's unit total is divided by the combined total of both drugs and multiplied by one hundred (Table 1).

Data

The data obtained in making percentage determinations of admixtures of powdered spearmint and peppermint are tabulated in Table 1. The experimentally determined percent (column 12) obtained by the use of the method described is tabulated along with the actual percentages, in the admixtures, of each of the drugs (column 13). The percentage variation is obtained by dividing the difference between the actual and calculated percentages by the actual percentage and multiplying by one hundred (column 14).

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SELECTED ABSTRACTS

The use of Mercurial Diuretics in the Treatment of Bromide Intoxication. Hussar, A. E., and Holley, H. L. *Am. J. Med. 20*:100 (1956). It had been demonstrated previously that the administration of large amounts of chloride will not only increase the total halide excretion but also the bromide fraction in the urine. It has been postulated that chloride ions are preferentially excreted by the kidney because the bromide ions move across the membranes more rapidly than do the chloride ions. The bromide ions are, therefore, more quickly re-absorbed in the tubules. Since the mercurial diuretics primarily affect the tubular epithelium, rendering the reabsorption of water and salts difficult, it seemed logical to anticipate that they would aid in increasing the excretion of bromide ions.

The authors studied the effects of mercuhydrin in conjunction with sodium chloride and ammonium chloride on the excretion of bromide from 11 patients hospitalized for chronic bromide intoxication.

It was found that mercuhydrin combined with sodium chloride caused an average increase in renal bromide excretion of 60 per cent. Combined with ammonium chloride, the average increase was 130 per cent. In the 9 patients treated with mercuhydrin and sodium or ammonium chloride, the serum bromide level decreased from an average of 236 mg. per cent to 75 mg. per cent in 9 days. The symptoms of bromide intoxication disappeared by the time the blood bromide level reached 100 mg. per cent.

The authors also found that the bromide output was independent of the urinary volume, that sodium chloride given alone was often more effective than ammonium chloride alone, but they were unable to demonstrate that the urinary bromide-chloride ratio was increased by mercurial diuretics.

The authors suggested that the combination of a mercurial diuretic with ammonium chloride is the most effective method available for increasing the elimination of bromides from the body. They suggested the following treatment schedule: ammonium chloride, orally, in a dose of 6 Gm. a day in divided doses with 2 ml., intramuscularly, of mercuhydrin every second or third day.

Salicylate Therapy in Recurrent Urolithiasis. Prien, E. L., and Walker, B. S. *J. A. M. A. 160*:355 (1956). In North America, about 90 per cent of all urinary calculi are calcium-containing. They usually are composed of calcium phosphate, calcium oxalate or magnesium ammonium phosphate. It has been shown that glucuronides significantly increase the solubility of calcium phosphate. Normally, glucuronides are present in small quantities in the urine. Their excretion can be increased by administering compounds which are excreted as conjugates of glucuronic acid. Salicylates are such compounds.

The authors found that the administration of acetylsalicylic acid in daily doses of 2 Gm. increased the glucuronide content of the urine from 2 to 4 times. Later, they found that salicylamide in the same dosage provided a higher excretion of glucuronides. Salicylamide was then used exclusively.

This treatment was given to 19 selected patients who had recurrent urinary calculi. Some had existing calculi while others had had a stone episode within a year. In addition to the above therapy, a mildly restricting diet was prescribed, principally restricting the ingestion of calcium. This treatment was continued for 18 to 24 months.

It was found that, during the period of treatment and observation, 17 of the 19 patients had no recurrence of stone formation and no increase in the size or density of existing stones. Another striking finding was with 9 patients requiring inlying urethral catheters. These catheters normally become obstructed with encrustations within 1 to 2 weeks. During the above treatment, this was completely prevented in 8 of the patients.

The authors concluded that the use of salicylates in preventing the recurrence of calcium-containing urinary calculi is promising therapy. However, further proof is necessary.

The Preservation of Poliomyelitis Vaccine with Ethylmercurithiosalicylate Sodium. Davisson, E. O., Powell, H. M., MacFarlane, J. O., Hodgson, R., Stone, R. L. and Culbertson, C. G. *J. Lab. Clin. Med. 47*:8 (1956). Poliomyelitis vaccines which had been preserved with ethylmercurithiosalicylate sodium (Merthiolate) were found to show deterioration in antigenicity. This situation was investigated by the authors.

It soon became evident that it was not the Merthiolate molecule but degradation products of it which were causing the reduction in

antigenicity. It had been known that very small traces of cupric ion in solution catalyzed the oxidation of Merthiolate. The presence of proteinaceous matter could be expected to adsorb trace amounts of heavy metals, but the finished poliomyelitis vaccine had a very low nitrogenous content. Therefore, ethylene diamine tetra-acetic acid (EDTA) was added to chelate the cupric ion.

By means of accelerated aging tests at 37° C., it was found that EDTA in a ration of 10 moles to 1 mole of Merthiolate prevented the deterioration of antigenicity in the vaccine as noted when Merthiolate was used alone. However, if all of the excess formaldehyde was not neutralized with sodium bisulfite, there was a loss in antigenicity. It was thought that this may be due to a sensitization of the virus particle to formaldehyde by the low concentration of ($C_2H_5Hg^+$) ethyl mercuri ion, the actively antiseptic ion from the Merthiolate reservoir. Therefore, it is essential that all of the excess formaldehyde be neutralized. Formaldehyde itself will also reduce the antigenicity of the vaccine over a period of time.

The authors also found that EDTA did not reduce the antibacterial or antifungal activity of Merthiolate in the vaccine. They reported that the finished vaccine contains a 25-fold excess of EDTA over the minimum required, in order to insure against the degradation of Merthiolate in the vaccine.

The Activity of Mixtures of Antibacterial Substances. Bavin, E. M., Kay, E., and Simmonite, D. *J. Pharm. Pharmacol.* 7:676 (1955). Preliminary studies on the possibility of combining more than one antiseptic substance in order to obtain greater effectiveness had shown that combinations of phenolic compounds exhibited no evidence of synergism. Attention was then directed to the quaternary ammonium compounds. Comparative studies on a number of bactericidal substances showed that Domiphen Bromide (alkyl dimethyl-2-phenoxy ethyl ammonium bromides) was the most active of the quaternary ammonium compounds tested against seven organisms. It was also found that 5-aminoacridine showed promise of acting as a reinforcing agent. The two substances were found to be compatible over a wide range of temperatures and, also, of concentrations.

When a concentration of 1 per cent domiphen bromide and 0.1 per cent 5-aminoacridine were combined it was found that under normal circumstances the combination possessed essentially the same antibacterial activity as domiphen bromide alone. However, in the presence of high concentrations of soap (0.05 per cent) and of blood (10 per cent) the 5-aminoacridine played an additive role. The antibacterial activity of the domiphen bromide was reduced but the activity of the 5-aminoacridine was not impaired and maintained the antibacterial activity of the mixture at a high level.

Preliminary studies using *Serratia marcescens* as the test organism indicated that the antiseptic mixture probably delayed the emergence of bacterial resistance *in vitro*, to a much greater extent than did domiphen alone.

The Effectiveness of Booster Inoculations in Children Vaccinated with Poliomyelitis Vaccine. Brown, G. C. *Am. J. Pub. Health 45:*1401 (1955). During the 1954 field trials of the efficacy of poliomyelitis vaccination in certain localities in Michigan, a representative number of the children had serologic antibody level determinations performed before and after vaccination. The antigenicity of the vaccine had varied and, thus, the antibody level also varied. One year later, booster inoculations were given and serological determinations were made on 119 subjects, both before and after the booster inoculation.

It was found that, whether the initial antibody level had been high or low, there was a gradual decline in titer. The average decline appeared to be greater among those that had had the higher level initially. Following the booster inoculation there was a sharp rise in antibody titer. The rise was particularly sharp in those that had responded poorly after the initial vaccination.

In an additional group of children who were tested by a more sensitive serologic technique, similar results were obtained with an even more satisfactory response to the booster inoculation.

The authors concluded that, although there is difficulty in comparing results obtained in groups receiving different vaccines in different areas, the data are of value because they were obtained under actual field conditions and verify the beneficial effects of a booster inoculation one year following the initial vaccination for poliomyelitis.

BOOK NOTICES AND REVIEWS

Organic Syntheses. Volume 34. W. S. Johnson, Editor-in-Chief. John Wiley and Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1954. vi + 121 pp. Price $3.50.

Volume thirty-four of this annual work consists of thirty-three selected and proved methods for the preparation of organic intermediates. Interesting compounds such as: cetyl malonic ester, di-tert-malonate, hemimellitene, *o*-phthalaldehyde, tetralin hydroperoxide and *o*-xylylene dibromide are included. The index is cumulative for volumes thirty through thirty-four.

A. R. GENNARO

Organic Syntheses. Volume 35. T. L. Cairns, Editor-in-Chief. John Wiley and Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1955. vi + 122 pp. Price $3.75.

The thirty-fifth volume of this annually anticipated series contains thirty-five selected preparations of great usefulness to the organic chemist. In addition to the general synthetic methods of more well known compounds, five preparations of the chlostenone family are included. As in the past all procedures have been checked to insure optimum conditions and good yields. The index is cumulative for volumes thirty through thirty-five.

A. R. GENNARO

Actinomycetes and Their Antibiotics. By Selman A. Waksman and Hubert A. Lechevalier. Williams & Wilkins Co., Baltimore, Md., 1953. 246 pages. 6 x 9¼ x ¾".

This is "A guide to the classification and identification of Actinomycetes and their antibiotics". The book includes a Preface, two Parts ("The Actinomycetes" and "The Antibiotics"), References, and two Indexes—one to species of Actinomycetes and the other to antibiotics of Actinomycetes.

Part I deals with the classification of the Actinomycetes, and the species Streptomyces (147), Micromonospora (5), Thermo-

actinomyces (3), Nocardia (43), and Actinomyces (2). Complete morphology, biochemical data, and source of each organism is given.

Part II concerns itself with the identification of antibiotics of Actinomycetes. A description of each of the 109 antibiotics derived from the Actinomycetes lists for each the organism producing same, method of extraction, chemical and physical properties, biological activity, toxicity, utilization, and references.

The text is indeed an important, enlightening, and valuable taxomony of the Actinomycetes and the antibiotics produced by them.

BERNARD WITLIN

The Quantitative Analysis of Drugs. 2nd ed. By D. C. Garratt. Philosophical Library, Inc., New York, 1955. xv + 670 pages. $17.50.

The first edition of this book, published in 1937 under the title *Drugs and Galenicals: Their Quantitative Analysis,* represented one of the most useful of collections of methods of assay of medicinals. This second edition is an expansion of the original work, with many monographs on the assay of newer drugs having been added. The author states that "it is of considerable satisfaction that only a small part of the original text could be deleted".

The subject matter of 524 pages of the book is comprised of monographs, arranged alphabetically, on analysis of drugs from acetanilid to zinc salts. A 30-page section on Oils, Fats and Waxes, another of 41 pages on Essential Oils and Drugs Containing Essential Oils, and an all-too-brief section of 9 pages on the general application of Physical Methods follow. Thirteen appendices, totaling 35 pages, deal with determinations of water, of alcohol content, of traces of metallic impurities in organic matter, and with extraction of non-volatile organic chemicals from viscera and vomits; provide a note on the determination of alkaloids, some hints on elimination of emulsions during extraction with immiscible liquids, micro-analytical tests for presence of extraneous matter in foods and drugs, a general discussion of titration in non-aqueous solvents, and various tables.

While this book is not all-inclusive in the field of analysis of medicinals it will be useful, and should be available, to all concerned with the subject.

ARTHUR OSOL

Industrial and Manufacturing Chemistry—Part I—Organic. Seventh Edition. By Geoffrey Martin. Philosophical Library, Inc., New York 16, New York, 1955. 752 pages, including Index. $17.50.

This volume is divided into twenty-four sections, each of which is written by a specialist in the subject in question. Apparently, most sections have been revised or implemented by the editor of this revision, Edward I. Cooke, or some of his assistants. The section on "The Plastics Industry" has been written especially for this edition and replaces "The Modern Explosives Industry".

Many of the references appear quite old, especially for such subjects as Petroleum Refining and Synthetic Rubber.

There is much factual material that will be of value to analytical and consulting chemists, such as tables of properties. On the other hand, there is little to recommend the volume to the designer of a chemical plant, mainly because much of the description of plant equipment is out-of-date.

There are very interesting sections on paper, sugar, starch, fermentation products, wood distillation, rubber, dyes, ink, fuel gas, aliphatic chemicals, paint, plastics, leather, glue, and photographic chemicals. The methods described in many cases are more European than American.

This volume may be considered as more in the nature of an encyclopedia of industrial chemistry, than as a text book for students of chemistry and chemical engineering. The editors have directed their efforts to prepare material for readers of such widely different training as chemists, technical lawyers, and inventors.

F. A. Fletcher

Atomic Energy Research at Harwell. By K. E. B. Jay. Philosophical Library, Inc., New York, 1955. xii + 144 pages; 19 half-tones. $4.75.

Harwell, in England, is the location of the famed British Atomic Energy Establishment, world-renowned for its research in various aspects of nuclear ("atomic") energy. The work done there between 1946 and 1951 was reported in 1952 in the book *Harwell—The British Atomic Energy Establishment, 1946-1951.* The book reviewed here describes principally the work performed between 1951 and 1954 and also, by virtue of some relaxation of regulations concerning the

release of secret information, certain details of subjects described in general terms in the first book.

This later book is divided into two parts, the first discussing major programs in terms understandable to non-technical readers, the second dealing in greater detail with selected researches and presented at a level understandable to scientific readers who are not specialists in the specific areas discussed. Included in Part I are chapters on Advances in the Production Program (of uranium and metallic fuels), Advances in the Reactor Program, Advances in the Isotope Program, The Development of Electronic Instruments, Some Special Techniques, Extra-mural Research, and Engineering and Allied Services. Part II includes chapters on Research in the Physics Divisions, Research in the Chemistry and Chemical Engineering Divisions, and Research in the Metallurgy Division. A glossary of technical terms is also provided.

This interesting book clearly indicates, both to non-technical and scientific readers, how far advanced are many phases of nuclear ("atomic") research and especially how universal the knowledge in this field has become.

ARTHUR OSOL

The Roger Adams Symposium. John Wiley and Sons, Inc., 440 Fourth Avenue., New York 16, N. Y. 1955. vii + 140 pp. Price $3.75.

This book is a record of the symposium held in honor of Dr. Roger Adams at the University of Illinois, September 3 and 4, 1954. It consists of a well written biography of Roger Adams by Ernest H. Volwiler and five excellent papers by Dr. Adams' former students:—Steric Effects in Dyes, by Wallace R. Brode; The Structure of Gliotoxin, A Sulfur Containing Antibiotic Substance, by John R. Johnson; The Structure of Nepatalic Acid, by S. M. McElvain; Chemistry of Flavillium Salts. Reactions with Amines, by Ralph L. Shriner; and Some Chemical Studies on Viruses, by Wendell M. Stanley. With each paper are many charts, tables and illustrations. Except for the paper on viruses there is also a reference list for each topic. The Roger Adams Symposium is not only an impressive tribute to a great scientist, but a concise resume of five subjects that makes very pleasant reading.

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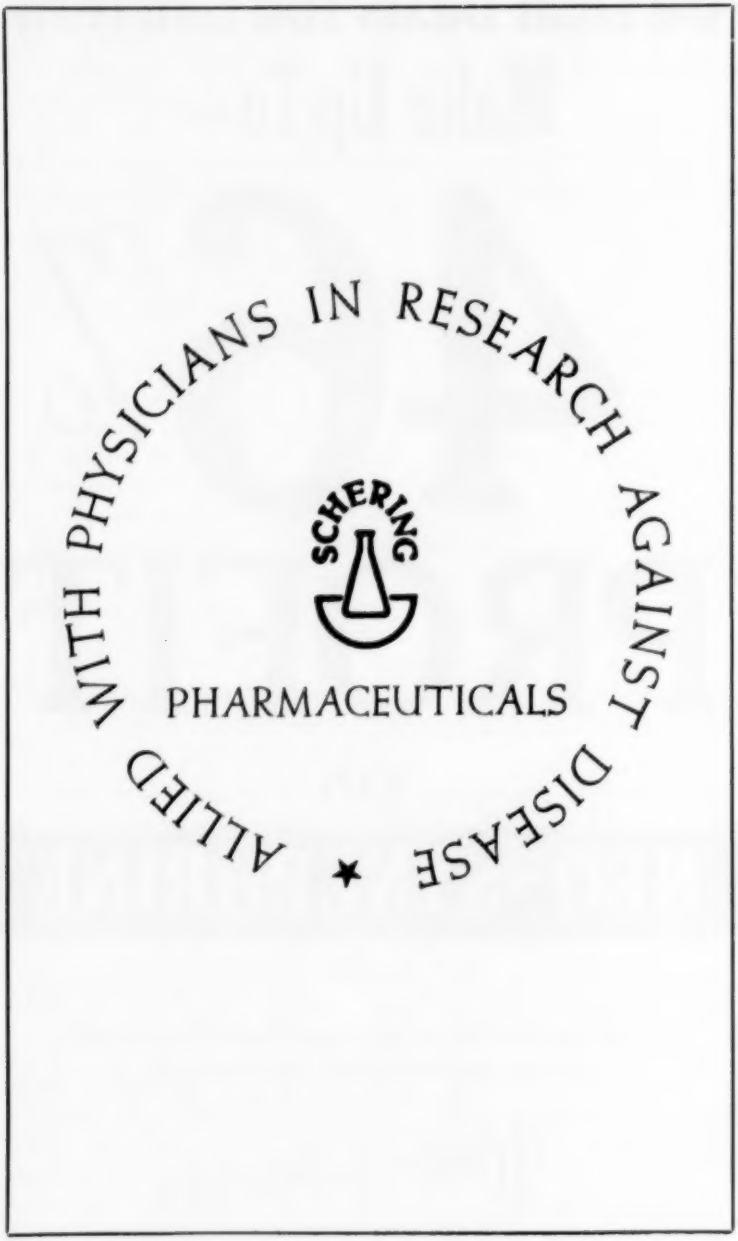
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